Pesticide Analysis in Foodstuffs, in Stomach Contents, and/or Liver Tissue

1 Introduction

This procedure is used to identify pesticides that are insecticides or herbicides.

2 Scope

This procedure allows for the screening and confirmation of common pesticides in a variety of foodstuffs, in stomach contents, or in liver tissue. This document applies to Chemistry Unit case working personnel who perform toxicology analyses and general chemistry analyses.

3 Principle

Most of the pesticides analyzed by this procedure will be classified as an insecticide or herbicide. The presence of chlorine or phosphorus in many of the chemicals within these classes permits the use of a gas chromatograph equipped with selective detectors (electron capture and nitrogen phosphorus). To be detected by this procedure, the pesticide must have some degree of solubility in n-hexane (foodstuffs and stomach contents extraction). The identification of a pesticide is achieved by use of the orthogonal technique of gas chromatography with mass spectrometry operated in the full scan electron ionization mode.

4 Specimens

Sample matrices typically comprise common foodstuffs, stomach contents, or liver tissue. Typically, 2.5 grams or 2.5 mL of sample are used for this analysis.

5 Equipment/Materials/Reagents

a. Gas Chromatograph capable of dual simultaneous injection and equipped with a 30-meter Rtx-CLPesticides column or equivalent connected to an electron capture detector (ECD) and a 30-meter Rtx-1701 column or equivalent connected to a nitrogen-phosphorous detector (NPD)

b. Gas Chromatograph/Mass Spectrometer equipped with a 30-meter DB-5 capillary column or equivalent

c. 5 N Sulfuric Acid (H_2SO_4)

d. n-Hexane 95% (HPLC, spectrophotometry, or gas chromatography grade or better)

e. Toluene (HPLC grade or better)

f. Deionized Water

g. Supported Liquid Extraction (SLE) cartridges (Biotage Isolute SLE+2; 2 mL sorbent mass; part number 820-0290-D)

h. Dichloromethane (Optima grade or better)

i. Acetone (HPLC grade or better)

g. Routine laboratory supplies, including homogenizer and/or mortar and pestle, test tubes (16 x 125 mm screw-top, 16x100 mm culture, 12 x 75 mm culture), centrifuge, rotator, vortex mixer disposable glass pipets, pH paper, spatulas, scalpels, test tube racks, graduated cylinders, vacuum extraction box, heated evaporator, etc.

6 Standards and Controls

a. Organochlorine (OC) Pesticides Stock Solution[1]:
A hexane:toluene (1:1) solution approximately 1 mg/mL each of aldrin, 4,4'-DDT, endrin, endrin aldehyde, and lindane (gamma BHC). Purchased as a special order item from Chemservice, Inc. Store refrigerated in glass. Stable for at least two years, or as determined by manufacturer.

b. Electron Capture Detector (ECD) Pesticides Testmix Solution[2]:
Dilute 25 µL of the OC pesticides stock solution to 50 mL with hexane, yielding a solution approximately 0.5 µg/mL in each component. Store refrigerated in glass. Stable for at least two years. A portion of this testmix is analyzed prior to each batch of Gas Chromatography – Electron Capture Detection (GC-ECD) analyses.

c. Organophosphate (OP) Pesticides Stock Solution[1]:
A hexane solution approximately 1 mg/mL each in chlorpyrifos, diazinon, fenchlorphos, parathion (ethyl), and prophos. Purchased as a special order item from Chemservice, Inc. Store refrigerated in glass. Stable for at least two years, or as determined by manufacturer.

[1] Standards can be individually purchased from Chemservice, Inc. or an equivalent supplier and prepared to an appropriate concentration in house.
[2] 100 µg/mL stock standards can be individually purchased from Chemservice, Inc. or an equivalent supplier and diluted to an appropriate concentration in house.

d. Nitrogen Phosphorus Detector (NPD) Pesticides Testmix Solution[2]:
Dilute 500 µL of the OP pesticides stock solution to 25 mL in hexane, yielding a solution approximately 20 µg/mL in each component. Store refrigerated in glass. Stable for at least two years. A portion of this testmix is analyzed prior to each batch of Gas Chromatography – Nitrogen Phosphorus Detection (GC-NPD) analyses.

e. Carbamate Pesticides Stock Solution[1,3]:
An acetonitrile solution approximately 1 mg/mL each in carbaryl, carbofuran, and propoxur. Purchased as a special order item from Chemservice, Inc. Store refrigerated in glass. Stable for at least six months, or as determined by manufacturer.

f. Hexachlorobenzene (98% or better purity):
Obtained as a solid from Sigma-Aldrich or an equivalent supplier. Storage and stability determined by manufacturer.

g. Triphenylphosphate (98% or better purity):
Obtained as a solid from Sigma-Aldrich or an equivalent supplier. Storage and stability determined by manufacturer.

h. 4-Bromo-3,5-dimethylphenyl-N-methylcarbamate (BDMC) (98% or better purity):
Obtained as a solid from Sigma-Aldrich or an equivalent supplier. Storage and stability determined by manufacturer.

i. Internal Standards Working Solution:
Weigh approximately 25 mg each of hexachlorobenzene, triphenylphosphate, and BDMC into a 25-mL volumetric flask and fill to the mark with toluene. Mix well and store refrigerated in glass. Stable for at least one year.

j. Pesticides GC-MS Testmix Solution:
Dilute 100 µL of the internal standards working solution to 10 mL in hexane, yielding a solution approximately 10 µg/mL in each component. Store refrigerated in glass. Stable for at least one year.

k. OC Pesticides Mix AB#1:
Obtained from Restek Corporation (Catalog #32291). This mixture contains twenty common organochlorine pesticides (aldrin, alpha-BHC, beta-BHC, delta-BHC, gamma-BHC, cis-chlordane, trans-chlordane, 4,4'-DDD, 4,4-'DDE, 4,4'-DDT, dieldrin, endosulfan I, endosulfan II, endosulfan sulfate, endrin, endrin aldehyde, endrin ketone, heptachlor, heptachlor epoxide (isomer B), and methoxychlor. Store refrigerated in glass. Stability determined by manufacturer.

l. OC Pesticides Retention Time Calibration Mix:
Dilute 20 µL of the OC Pesticide Mix AB#1 to 10 mL in hexane. Store refrigerated in

[3] Dilute stock solution prepared in house with acetone instead of acetonitrile.

glass. Stable for at least two years. A portion of this calibration mix should be analyzed by GC-ECD at least every six months or with every case batch, whichever is longer, to check retention time stability. Maintain a record of these samples with the instrument testmix records.

m. OP Pesticides Mix B:
Obtained from Restek Corporation (Catalog #32278). This mixture contains seven common organophosphate pesticides (tetraethylpyrophosphate (TEPP), sulfotepp, monocrotophos, dimethoate, malathion, parathion, and EPN). Store refrigerated in glass. Stability determined by manufacturer.

n. OP Pesticides Retention Time Calibration Mix:
Dilute 100 µL of the OP Pesticides Mix B to 500 µL with hexane. Store refrigerated in glass. Stable for at least two years. A portion of this calibration mix should be analyzed by GC-NPD at least every six months or with every case batch, whichever is longer, to check retention time stability. Maintain a record of these samples with the instrument testmix records.

o. Negative Control:
A deionized water blank or a matrix similar to the submitted specimen (if known and available) is used as the Negative Control. A negative control is extracted and analyzed with every assay.

p. Positive Control Foodstuff or Stomach Contents:
The suggested Positive Control is a spiked aliquot of the questioned sample. If sample volume is limited, a blank matrix similar to the submitted specimen may be used, or, if that is unobtainable, deionized water may be spiked to create a positive control. Alternatively, if a specific pesticide is being targeted it is acceptable to prepare a 10 µg/mL solution of the targeted pesticide in a blank matrix or within deionized water. To prepare: add 25 µL each of the OC Pesticides Stock Solution (1 mg/mL), the OP Pesticides Stock Solution (1mg/mL), and the Carbamate Pesticides Stock Solution (1mg/mL) to 2.5 mL or 2.5 g of the sample matrix. This will yield a spiked sample that is approximately 10 µg/mL in each of the thirteen control analytes. A Positive Control is extracted and analyzed with every assay.

q. Positive Control Liver Tissue
To prepare: add 50 µL each of the OC Pesticides Stock Solution (1 mg/mL), the OP Pesticides Stock Solution (1mg/mL), and the Carbamate Pesticides Stock Solution (1mg/mL) to 5 g of the liver homogenate (2.5 g liver tissue: 2.5 g deionized water). This will yield a spiked sample that is approximately 20 µg/g in each of the thirteen control analytes. A Positive Control is extracted and analyzed with every assay.

7 Sampling

Not applicable.

8 Procedure

Appendix 1 contains an abbreviated version of this procedure. This form may be used at the bench by the examiner or chemist performing the procedure.

8.1 Foodstuff or Stomach Contents:

a. Photograph the specimen if its appearance will be grossly altered by homogenizing.

b. For liquid specimens, mix the specimen well, sample 2.5 mL into a screw-top tube, and mix this aliquot with 2.5 mL of deionized water.

c. For solid or semi-solid specimens, homogenize a portion of the specimen 1:1 with deionized water (2.5 g specimen: 2.5 g deionized water) into a screw-top tube. Homogenization may be accomplished by grinding in a mortar and pestle (e.g., candy), grinding by hand (e.g., cakes, cookies), or blending in a homogenizer (e.g., bulky solids or stomach contents).

d. Add 25 µL of the internal standards working solution[4] and vortex for 30 seconds.

e. Add 70 µL of 5 N H_2SO_4 and vortex for 30 seconds. Ensure the pH is less than 6 with indicator paper.

f. Add 5 mL of n-hexane and extract by rotation for 20 minutes. Centrifuge for 15 minutes at high speed. If a severe emulsion forms, stir the emulsion with a clean wooden stick and re-centrifuge.

g. Transfer the hexane supernatant to a 16x100 mm culture tube.

h. Mix a 200 µL portion of the extract with 800 µL of hexane and analyze 1 µL of this solution by GC-ECD.

i. Concentrate a 1.5 mL portion of the extract to ca. 250-300 µL under a stream of nitrogen at ca. 40ºC. Analyze 1 - 2 µL portions of this concentrate by GC-NPD and GC-MS.

[4] Other internal standards may be substituted at relevant concentrations if deemed appropriate.

8.2 Liver Tissue

a. Weigh a portion of liver tissue (2.5 g)[5]. Place specimen and an equal amount of deionized water (2.5 g specimen: 2.5 g deionized water) into a homogenizer. Blend for 2-3 minutes on high. Transfer homogenate to a 16 x 100 mm culture tube with a polypropylene snap-top.

b. Add 50 µL of the internal standards working solution[6] and vortex for 30 seconds.

c. Centrifuge homogenate at high speed for 15 minutes. Transfer supernatant to a clean 12 x 75 mm culture tube with a polypropylene snap-top. Re-centrifuge supernatant at high speed for 15 minutes (repeat above transfer and centrifuge if supernatant contains any homogenate),

d. Load supernatant samples onto SLE cartridges by gravity. (A brief application of vacuum will be necessary to start loading.) DO NOT ELUTE.

e. Allow to stand for 5 minutes.

f. Apply 3 mL of Dichloromethane and allow to absorb.

g. Allow to stand for 5 minutes. DO NOT APPLY VACUUM.

h. Elute by gravity into 16 x 100 mm culture tubes with 2 x 4 mL Dichloromethane. Briefly apply full vacuum to complete elution.

i. Evaporate at approximately 45ºC. When eluent reaches 0.5 – 1 mL, briefly vortex before evaporating to dryness.

j. Reconstitute with 0.1 mL Acetone. Transfer 25 µL to properly labeled autosampler vials, add 225 µL Acetone, analyze 1 µL of this solution by GC-ECD. Transfer remaining extract to properly labeled autosampler vials, analyze 1 – 2 µL of this solution by GC-MS(EI). Note: No GC/NPD analysis will be conducted on liver tissue samples.

9 Instrumental Conditions

Following are the suggested instrumental parameters for the instruments used in this procedure. Appendix 2 contains an abbreviated version of this procedure. This form may be used at the bench by the examiner or chemist performing the procedure.

[5] Other amounts of liver homogenate may be prepared as long as a 1:1 ratio of liver :water is maintained. If the test sample amounts permits, spike a second portion with positive control solution.
[6] Other internal standards may be substituted at relevant concentrations if deemed appropriate.

9.1 Gas Chromatograph/Nitrogen Phosphorus Detector / Electron Capture Detector Parameters (Simultaneous Dual Injection)

Inlet Parameters - ECD		Inlet Parameters - NPD		GC Oven Parameters	
inlet temperature	230°C	inlet temperature	250°C	temperature 1	125°C
injection mode	splitless	injection mode	split	hold 1	1
carrier gas	helium	carrier gas	helium	ramp 1	7°C/min
carrier mode	constant pressure	carrier mode	constant pressure	temperature 2	280°C
carrier pressure	16.85 psi	carrier pressure	13.39 psi	hold 2	22 min
splitless time	0.5 min	split ratio	15:1	Column Parameters – ECD	
Detector Parameters - ECD		Detector Parameters - NPD		type	Rtx-ClPest
temperature	300°C	temperature	250°C	length	30 m
makeup gas	nitrogen	offset	10	inner diameter	0.32 mm
makeup flow	30 mL/min	makeup flow (nitrogen)	30 mL/min	film thickness	0.5 µm
		air flow	60 mL/min	Column Parameters - NPD	
		hydrogen flow	2 mL/min	type	Rtx-1701
				length	30 m
				inner diameter	0.32 mm
				film thickness	0.5 µm

9.2 Gas Chromatograph / Mass Spectrometer

Oven Parameters		Inlet and Carrier Parameters		Column Parameters	
temperature 1	60°C	inlet temperature	220°C	type	DB-5MS
hold 1	3.2 min	injection mode	split	length	30 m
ramp 1	35°C/min	carrier gas	ultrapure helium	internal diameter	0.25 mm
temperature 2	280°C	carrier mode	constant flow	film thickness	0.25 µm
hold 2	31 min	carrier flow	1.2 mL/min		
total run time	40.5 min	split flow	12 mL/min		
		split ratio	10:1		

Mass Spectrometer Parameters			
ionization mode	electron impact (+)	source temperature	230°C
scan mode	full scan	transfer line temperature	280°C
scan range	35 – 500 AMU	solvent delay	5 min

10 Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay. In most cases, all of the below should be met in order to identify a pesticide within a foodstuff or gastric content sample.

10.1 Chromatography

The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. In order to be determined acceptable, a chromatographic peak in an unknown sample should compare favorably to a chromatographic peak of the same analyte in a known sample analyzed on the same system in the same or subsequent analytical runs. Additionally, the following two criteria should be met.

10.1.1 Retention Time

The retention time of the peak should be within ± 2% of the retention time (relative or absolute, as appropriate) obtained from injection of a reference standard or an extracted positive control

10.1.2 Signal-to-Noise

To justify the existence of a peak, its baseline signal noise ratio should exceed 3. Further, the baseline signal for the peak of interest should be at least 10 fold greater than that for any observed peak at similar retention time in a Negative Control or blank solvent injected just prior to the sample.

10.2 Mass Spectrometry

The mass spectrum of the analyte of interest should compare favorably with that of a reference standard, or an extracted Positive Control. See the Guidelines for Comparison of Mass Spectra standard operating procedure (Tox 104) for further guidance.

11 Calculations

Not applicable.

12 Measurement Uncertainty

Not applicable.

13 Limitations

a. Limit of Detection: The limit of detection varies depending on the pesticide of interest, and the matrix being analyzed. All thirteen target analytes contained in the pesticides stock solutions used in this procedure can be detected at levels of at least 10 µg/mL in a wide variety of food matrices. The limits of detection for pesticides in liver tissue are listed below in Table 1.

Table 1; LOD Pesticides in Liver Tissue

	GC-ECD	GC-MS
Organochlorines		
Lindane (g-BHC)	5 µg/g	5 µg/g
Aldrin	5 µg/g	5 µg/g
Endrin	5 µg/g	10 µg/g
Endrin Aldehyde	5 µg/g	10 µg/g
4,4-'DDT	5 µg/g	5 µg/g
Carbamates		
Carbaryl		5 µg/g
Carbofuran		5 µg/g
Propoxur		5 µg/g
Organophosphates		
Chlorpyrifos	10 µg/g	5 µg/g
Diazinon		5 µg/g
Fenchlorphos		5 µg/g
Parathion		5 µg/g
Profos		10 µg/g

b. Interferences: None known. Grossly decomposed or putrefied samples may affect both detection.

c. Other Considerations: Carbamate pesticides can undergo chemical breakdown to the corresponding phenolate compounds when subjected to GC injection. The extent of this breakdown depends upon sample matrix, analyte loading, and the age and condition of the GC injection port liner. For any carbamate pesticide, the presence of the phenolate breakdown product may be considered as evidence of the presence of the parent compound if the breakdown product is also observed in a contemporaneously analyzed matrix-matched positive control specimen. This procedure is not suitable for the detection of aldicarb and its metabolites.

14 Precautionary Statement

Care should be taken in the interpretation of pesticide levels in foodstuffs. Some pesticides have a legitimate use on food products and a qualitative identification in the absence of quantitative

data could produce confusion in interpreting results. The meaning of the toxicological significance of a negative pesticide finding should be considered in conjunction with its biodegradability. Exercise care in reporting and interpreting all pesticide results.

15 Safety

Take standard precautions for the handling of chemicals and biological materials. It should be noted that many of the pesticides detected by this procedure may be extremely toxic and/or carcinogenic. The utmost caution should be taken in handling reference materials and case specimens containing such pesticides. Refer to the *FBI Laboratory Safety Manual* for guidance.

This procedure utilizes the following P-listed pesticides: aldrin, carbofuran, dimethoate, endosulfan, endrin & metabolites, heptachlor, parathion, and tetraethylpyrophosphate (TEPP). This SOP utilizes the following U-listed pesticides: carbaryl, DDD, DDT, hexachlorbenzene, methoxychlor,and propoxur.

16 References

Clarke's Isolation and Identification of Drugs; *Pesticides*. Second Edition. The Pharmaceutical Press. London, 1986. pp 70-86.

Farm Chemicals Handbook; Meister Publishing Company. Willoughby, Ohio. published annually.

Mass Spectral Data Compilation of Pesticides and Industrial Chemicals; Los Angeles District Laboratory; Mass Spectrometry Service Center; Los Angeles, California. 1987.

Mass Spectrometry of Pesticides and Pollutants; CRC Press. Cleveland, Ohio. 1973.

Guidelines for Comparison of Mass Spectra (Tox 104); FBI Laboratory Chemistry Unit – Toxicology SOP Manual.

FBI Laboratory - Instrument Operation and Support SOP Manual.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
3	08/05/19	Document was discontinued between 08/21/2018 – 08/04/2019. Updated Introduction language, Scope language and added General Chemistry. Updated wording Section 3, 4, 10, 11, 14. Section 5 updated reagent grades. Section 6 updated standards and controls. Section 7 Calibration – removed and renumbered Sections 7 – 16. Section 8 Procedure converted drops to microliters for sulfuric acid and increased injection volume. Section 9 updated typo in gc/ms scan range. Section 11 and 12 updated to not applicable. Section 15 Safety added P-listed and U-listed pesticides utilized in this procedure. Removed "reasonable degree of scientific certainty" language from Section 11.2. Updated approval lines. Removed references no longer needed.
4	09/11/19	Updated Title. Updated Section 2, 3 and 4 to include liver tissue. Added equipment, reagents and supplies needed to extract liver tissue in Section 5. Revised Positive Control scheme in Section 6 p and q. Modified procedure 8.1 for Foodstuff or Stomach Contents and 8.2 for Liver Tissue. Section 13 a added LOD for pesticides in liver tissue and updated 13c statement re: aldicarb.

Approval

Redacted - Signatures on File

Acting Toxicology Technical Leader: _____ Date: 09/09/2019

General Chemistry Technical Leader: _____ Date: 09/09/2019

Chemistry Unit Chief: _____ Date: 09/09/2019

QA Approval

Quality Manager: Date: 09/09/2019

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Appendix 2: Abbreviated version of the Instrumental Parameters for bench use.

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